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Award Number: W81XWH-10-1-0872

TITLE: Inhibition of Orthopaedic Implant Infections by Immunomodulatory Effects of Host Defense Peptides

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REPORT DATE: October 2011

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

# REPORT DOCUMENTATION PAGE

*Form Approved*
*OMB No. 0704-0188*



| 1. REPORT DATE *(DD-MM-YYYY)* | 2. REPORT TYPE | 3. DATES COVERED *(From - To)* |
|---|---|---|
| 01-10-2011 | Annual | 15 Sep 2010 - 14 Sep 2011 |

**4. TITLE AND SUBTITLE**
Inhibition of Orthopaedic Implant Infections by Immunomodulatory Effects of Host Defense Peptides

**5a. CONTRACT NUMBER**

**5b. GRANT NUMBER**
W81XWH-10-1-0872

**5c. PROGRAM ELEMENT NUMBER**

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**5d. PROJECT NUMBER**

**5e. TASK NUMBER**

**5f. WORK UNIT NUMBER**

**7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)**
Case Western Reserve University
Cleveland, OH 44106

**8. PERFORMING ORGANIZATION REPORT NUMBER**

**9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)**
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

**10. SPONSOR/MONITOR'S ACRONYM(S)**

**11. SPONSOR/MONITOR'S REPORT NUMBER(S)**

**12. DISTRIBUTION / AVAILABILITY STATEMENT**
Approved for Public Release; Distribution Unlimited

**13. SUPPLEMENTARY NOTES**

**14. ABSTRACT**

Host defense peptides represent a promising new approach to inhibit infection. The anti-infective actions of these peptides are primarily due to their immunomodulatory effects. Since they regulate multiple aspects of the mammalian immune system, host defense peptides are also less likely to induce bacterial resistance than are traditional antibiotics. The local delivery of anti-bacterial agents allows for both high local concentrations to increase efficacy and low systemic levels to reduce toxicity. A promising strategy for the local delivery of anti-bacterial agents is to bind them directly to the surfaces of orthopaedic implants. The progress in the first year of this project has created a strong foundation for more rapid progress in the subsequent years to test our hypotheses that: 1. Soluble host defense peptides reduce infection of orthopaedic implants. 2. Host defense peptides bound to orthopaedic implant surfaces reduce infection. The host defense peptides have the potential to substantially reduce infections of fractures sustained on the battlefield and in civilian settings. If the synthetic peptide reduces infections in the studies proposed in this application, more extensive pre-clinical testing would precisely determine its potential benefits and risks and determine whether the peptide is a high priority for human trials.

**15. SUBJECT TERMS**
Orthopaedic infections, Host Defense Peptides, Murine model, Staphylococcus aureus, Acinetobacter baumannii

| 16. SECURITY CLASSIFICATION OF: | | | 17. LIMITATION OF ABSTRACT | 18. NUMBER OF PAGES | 19a. NAME OF RESPONSIBLE PERSON |
|---|---|---|---|---|---|
| a. REPORT | b. ABSTRACT | c. THIS PAGE | | | USAMRMC |
| U | U | U | UU | 7 | 19b. TELEPHONE NUMBER *(include area code)* |

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INTRODUCTION:

Host defense peptides represent a promising new approach to inhibit infection. The anti-infective actions of these peptides are primarily due to their immunomodulatory effects. Since they regulate multiple aspects of the mammalian immune system, host defense peptides are also less likely to induce bacterial resistance than are traditional antibiotics. The local delivery of anti-bacterial agents allows for both high local concentrations to increase efficacy and low systemic levels to reduce toxicity. A promising strategy for the local delivery of anti-bacterial agents is to bind them directly to the surfaces of orthopaedic implants. Our hypotheses are:

1. Soluble host defense peptides reduce infection of orthopaedic implants.
2. Host defense peptides bound to orthopaedic implant surfaces reduce infection.

BODY:

**Aim 1: Test the hypothesis that soluble host defense peptides reduce infection of orthopaedic implants.**

Task 1: Establish a murine model of implant infection

**Milestone #1: Obtain animal use approval:** Completed

**Milestone #2: Select bacteria doses and number of mice per group to use in Tasks 2 & 5:** We have developed methods to reproducibly and quantitatively add known amounts of bacteria to the implants (Fig. 1). Animal experiments with known amounts of bacteria on the implants will begin in the near future.

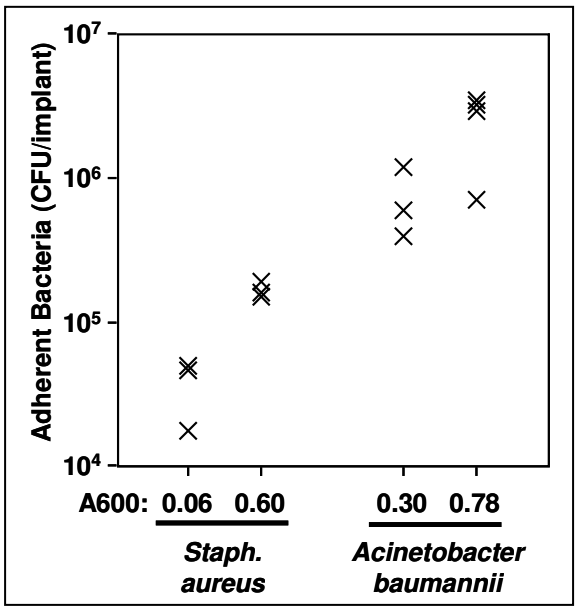


**Figure 1.** Adherence of bacteria to implants. Cultures of *Staphylococcus aureus* or *Acinetobacter baumannii* with indicated absorbance values (A600) were incubated with implants for 20 minutes. Adherent bacteria were removed by sonication and quantified by measuring colony forming units (CFU) on LB broth plates. Each "X" represents an individual implant

Task 2: Determine whether soluble host defense peptides reduce implant infection

**Milestone #3: Determine whether soluble host defense peptides reduce implant infection:** We have obtained IDR-1 & the inactive analogue and shown that there is no detectable LPS in the peptide preparations (Subtask 2A). Experiments to confirm the in vitro activity of the peptides are on-going (Subtask 2B) and in vivo IDR-1 experiments will begin in the near future (Subtasks 2C-D).

**Milestone #4: Report(s)/manuscript(s) with results from Tasks 1-2:** Reports/manuscripts will be prepared upon completion of Tasks 1-2.

**Aim 2: Test the hypothesis that host defense peptides bound to orthopaedic implant surfaces reduce infection.**

Task 3: Determine which binding motifs/domains bind to implant surfaces

**Milestone #5: Select binding motifs for study in Task 4**: We have obtained peptides with titanium binding motifs and demonstrated that they bind with high affinity to titanium particles (Fig. 2).

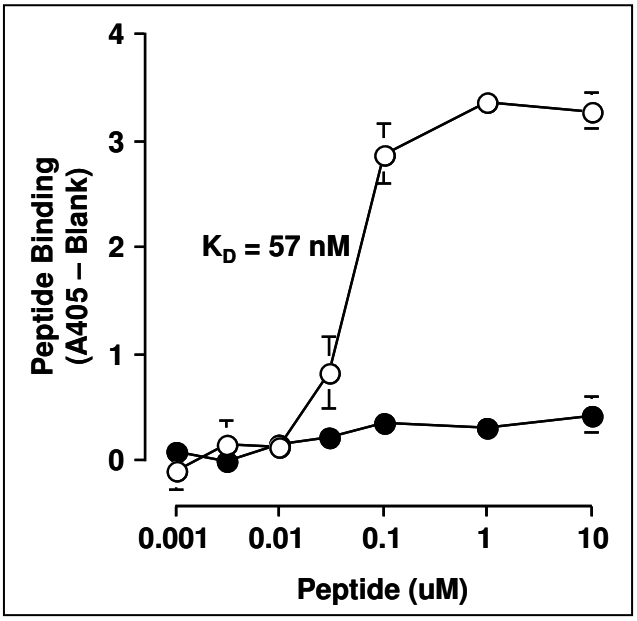


**Figure 2.** Peptide binding to titanium alloy particles. Biotinylated-3xHKH peptide (open circles) or non-binding control peptide (filled circles) were incubated with titanium alloy particles for 1 hour and unbound peptide removed by washing three times with PBS containing 0.5% Tween-20. Bound peptides were detected by incubation with streptavidin-alkaline phosphatase conjugate followed by measurement of bound alkaline phosphatase activity. Each symbol represents mean ± SEM (n=5).

Task 4: Determine whether host defense peptides bound to implant surfaces retain immunomodulatory activity.

**Milestone #6: Obtain approval to obtain human monocytes for cell culture experiments:** Completed

**Milestone #7: Determine whether bound host defense peptides retain substantial immunomodulatory activity and select fusion peptides for study in Task 5.** We have established protocols to measure expression by monocytes of chemokines/cytokines (Subtask 4B) and have obtained fusion peptides with IDR-1 & binding motifs/domains and shown that there is no detectable LPS in the peptide preparations (Subtask 4C). Experiments to measure effects of bound IDR-1 on expression of chemokines and cytokines are on-going (Subtask 4D).

**Milestone #8: Report(s)/manuscript(s) with results from Tasks 3-4:** Reports/manuscripts will be prepared upon completion of Tasks 3-4.

Task 5: Determine whether bound host defense peptides reduce implant infection.

**Milestone #9: Determine whether bound host defense peptides reduce implant infection:** These experiments will begin upon completion of Milestone #7.

**Milestone #10: Report(s)/manuscript(s) with results from Task 5:** Reports/manuscripts will be prepared upon completion of Task 5.

KEY RESEARCH ACCOMPLISHMENTS:

- Developed methods to reproducibly and quantitatively add known amounts of bacteria to the implants (Fig. 1).
- Demonstrated that peptides with titanium binding motifs bind with high affinity to titanium particles (Fig. 2).
- Demonstrated that the peptides preparations are not contaminated with LPS (Subtasks 2A & 4C).
- Established protocols to measure expression by monocytes of chemokines/ cytokines (Subtask 4B).

REPORTABLE OUTCOMES: None at this time

CONCLUSION:

The progress in the first year of this project has created a strong foundation for more rapid progress in the subsequent years to test our hypotheses that:

1. Soluble host defense peptides reduce infection of orthopaedic implants.
2. Host defense peptides bound to orthopaedic implant surfaces reduce infection.

The host defense peptides have the potential to substantially reduce infections of fractures sustained on the battlefield and in civilian settings. If the synthetic peptide reduces infections in the studies proposed in this application, more extensive pre-clinical testing would precisely determine its potential benefits and risks and determine whether the peptide is a high priority for human trials.

REFERENCES: Not applicable

APPENDICES: Not applicable